



Anti-Allergic Effect of a Hot-Water Extract of Quince (*Cydonia oblonga*)

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We examined the effect of a crude hot-water extract (HW) of quince (*Cydonia oblonga* Miller) fruit on type I allergy *in vivo* and *in vitro*. The oral administration of the quince HW-added diet to NC/Nga mice for 63 d showed a significant decrease in the development of atopic dermatitis-like skin lesions under conventional conditions. The concentration of IgE in the serum collected from mice fed with quince HW was also lowered in a dose-dependent manner. Moreover, we found that quince HW inhibited the release of β -hexosaminidase from rat basophilic leukemia cell line RBL-2H3 after a 24-h treatment. The quince HW fraction of less than 3 kDa reduced the mRNA expression of the high-affinity IgE receptor (Fc ϵ RI) γ subunit. These results suggest that quince HW had an inhibitory effect on type I allergy by suppressing IgE production and IgE-mediated degranulation.

Key words: quince; NC/Nga mouse; degranulation; IgE; high affinity IgE receptor (Fc ϵ RI)

Quince (*Cydonia oblonga* Miller) is a deciduous shrub originating in Central Asia. The fruit features a rich yellow and waxy surface. Although they are too hard to eat raw and have a strongly acidic and astringent taste, they exhibit an extremely pleasant aroma. Therefore, extracts of quince fruits have long been used for honeydew and liquors. With regard to the function of the quince fruit, a methanol extract and an aqueous acetone extract have been reported to exhibit antioxidative,¹⁻⁴⁾ antiviral,²⁾ antiulcerative,³⁾ and antimicrobial activities.⁴⁾ In addition, some products made from the hot-water extract (HW) of quince fruits are empirically known to be effective for treating sore throats and to relieve coughs. However, little is known about the effect of quince on such pathological symptoms.

Some sore throats and coughs are included in major allergic symptoms. Allergic reactions can be classified into four or, according to one current explanation, five types.^{5,6)} Among them, there has recently been an increasing incidence of type I allergic disorders from food- and air-borne allergens worldwide.^{7,8)} This type of allergy is recognized as an IgE-mediated immediate hypersensitive reaction against environmental allergens and is believed to be associated with an elevated level of IgE antibodies produced against the allergens.⁹⁾ Cross-linking of the high-affinity IgE receptor (Fc ϵ RI) on the

surface of mast cells or basophils by IgE and a multivalent antigen triggers the exocytotic release of many chemical mediators including histamine. These mediators cause an immediate allergic reaction.¹⁰⁾

Since these allergic reactions are caused by a series of events, the inhibition of many steps is considered to attenuate the allergic symptoms. In fact, many researchers have reported the distinct mechanism-based anti-allergic effect of fruits.¹¹⁻¹³⁾ Given the effectiveness of quince HW-supplemented products against sore throats and coughs, quince HW can also be expected to have some sort of inhibitory effect against the type I allergic process.

We examined in the present study the anti-allergic effects of quince HW *in vivo* and *in vitro*. To evaluate the effect *in vivo*, we investigated an oral administration of HW to atopic model mice. Furthermore, we evaluated the anti-degranulation effect of HW by using an *in vitro* culture of RBL-2H3 cells, frequently used as a model of mast cells and basophils for their availability in IgE-mediated degranulation.

Materials and Methods

Preparation of the quince sample. Quince fruits were purchased from a fruit and vegetable market in Minowa-machi. They were cut into approximate 10 × 10 mm-size pieces including the peel, pulp and seeds, and then boiled in hot water for 1 h. After filtering the extract through a layer of cloth, the filtrate was centrifuged at 5,000 × g for 30 min. The resulting supernatant was concentrated to 450 ml in an RE 400A-W rotary evaporator (Yamato, Tokyo, Japan) at 40 °C, and freeze-dried to obtain quince HW.

Fractionated quince HW was prepared by passing the HW solution through Vivaspin 500 apparatus (MW cut off of 3,000; Vivascience, Hannover, Germany). After centrifugation at 5,000 × g, the lower (<3,000) fraction was collected. The unpassed contents were obtained as >3,000 fraction by re-dissolving the same volume of DW.

Mice. Three-week-old male NC/Nga mice were purchased from Japan SLC (Shizuoka, Japan) and housed at 23 ± 3 °C under a 12-h light/dark cycle. All animal protocols used in this study were approved by the Committee for Animal Experiments of Shinshu University.

Feeding and sampling. The mice were acclimatized with free access to an AIN-93M standard rodent diet (Clea Japan, Tokyo, Japan) and water for 7 d under conventional conditions. They were then assigned three groups consisting of five mice each on the basis of their body weights and fed on the following diets *ad libitum* for 8 weeks: control diet (AIN-93M), 2.5% quince HW-supplemented diet, and 5.0% quince HW-supplemented diet (Table 1). Throughout the experimental

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Abbreviations: Fc ϵ RI, high-affinity IgE receptor; TNF, tumor necrosis factor; HW, hot-water extracts; AD, atopic dermatitis; ELISA, enzyme-linked immunosorbent assay; DNP, dinitrophenyl; RT-PCR, reverse transcription polymerase chain reaction; SD, standard deviation

Table 1. Composition of the Test Diets

Ingredient	Control diet (%)	2.5% quince HW-added diet (%)	5.0% quince HW-added diet (%)
Corn starch	46.57	46.57	46.57
Milk casein	14.00	14.00	14.00
α -corn starch	15.50	15.50	15.50
Granulated sugar	10.00	10.00	10.00
Purified soybean oil	4.00	4.00	4.00
Avicel (cellulose powder)	5.00	2.50	—
Quince HW extract	—	2.50	5.00
Mineral mix (AIN-93M-MX)*	3.50	3.50	3.50
Vitamin mix (AIN-93VX)**	1.00	1.00	1.00
L-cystine	0.18	0.18	0.18
Coline bitartrate	0.25	0.25	0.25
Tertiary butyl hydroquinone	0.00	0.00	0.00
Total	100.00	100.00	100.00

*Mineral mix: calcium carbonate anhydrous, 35.7%; potassium phosphate, monobasic, 25.0%; sodium chloride, 7.4% potassium sulfate, 4.66%; potassium citrate, tri-potassium, monohydrate, 2.8%; magnesium oxide, 2.4%; ferric citrate, 0.606%; zinc carbonate, 0.165%; manganese carbonate, 0.063%; cupric carbonate, 0.03% potassium iodate, 0.001%; sodium selenate anhydrous, 0.001025%; ammonium paramolybdate, 4 hydrate, 0.00795%; Sodium meta-silicate, 9 hydrate, 0.145%; Chromium potassium sulfate, 12 hydrate, 0.0275%; boric acid, 0.00815%; sodium fluoride, 0.00635%; nickel carbonate, 0.00318% lithium chloride, 0.00174%; ammonium vanadate, 0.00066%; powdered sucrose, 20.9806%

**Vitamin mix: nicotinic acid, 0.30%; Ca pantothenate, 0.32%; pyridoxine-HCl, 0.07%; thiamin-HCl, 0.06%; riboflavin, 0.06%; folic acid, 0.02%; D-biotin, 0.10%; vitamin B12, 0.25%; vitamin E, 1.50%; vitamin A, 0.04%; vitamin D3, 0.02%; vitamin K1, 0.0075%; powdered sucrose, 97.2525%

period, the body weights of the mice were recorded weekly. Blood samples were collected from the abdominal aorta of the mice under ether anesthesia on d 63 to measure the serum IgE concentration.

Assessment of the clinical skin severity. The clinical severity of atopic dermatitis (AD)-like skin lesions of the five signs and symptoms (erythema/hemorrhage, edema, hair loss, dryness, and scaling) of the mice was assessed every week. The total clinical score for the severity is defined as the sum of individual scores graded as 0 (subclinical), 1 (mild), 2 (moderate), 3 (severe), and 4 (very severe) as described previously.¹⁴ Additionally, photographs of representative mice exhibiting average clinical symptoms in each group were taken after the majority of mice had shown AD-like skin lesions.

Measurement of the IgE level. The serum total IgE level was measured by an enzyme-linked immunosorbent assay (ELISA). Briefly, blood samples collected in assist tubes were stored overnight at 4°C for blood clot formation. After clotting, the serum was separated by centrifugation at 500 × g for 30 min and stored at -80°C until needed. The IgE concentration of the serially diluted sera was measured with a mouse IgE ELISA kit (Bethyl Laboratories, Montgomery, TX) according to the manufacturer's protocol.

Cells. The rat basophilic leukemia cell line, RBL-2H3, was purchased from the Health Science Research Resources Bank (Osaka, Japan). The cells were maintained in an RPMI-1640 medium (Nissui, Tokyo, Japan) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Equitech-Bio, TX), 2 mM L-glutamine (Invitrogen, Carlsbad, CA), 100 IU/ml of penicillin (MP Biomedicals, Irvine, CA), and 100 µg/ml of streptomycin (Wako, Osaka, Japan) in a humidified atmosphere of 5% CO₂/95% air at 37°C.

Beta-hexosaminidase release assay. To evaluate IgE-mediated degranulation, a β -hexosaminidase release assay was employed as described previously.¹⁵ RBL-2H3 cells (0.5×10^6 cells/ml) were treated with quince HW at 0, 50, 100, and 200 µg/ml in a 35-mm

dish (Nunc, Roskilde, Denmark) for 24 h. After the treatment, the cells were harvested and washed twice with a HEPES-Tyrod buffer (137 mM NaCl, 5.6 mM glucose, 2.7 mM KCl, 0.5 mM NaH₂PO₄, 1.0 mM CaCl₂ and 10 mM HEPES at pH 7.3) containing 0.1% bovine serum albumin. The washed cells were suspended in the HEPES-Tyrod buffer and inoculated into a 96-well tissue culture plate (Becton Dickinson, NJ) at a concentration of 1×10^5 cells/ml. The cells were sensitized or not with 1 µg/ml of the mouse monoclonal anti-dinitrophenyl (DNP) IgE antibody (clone SPE-7; Sigma, St. Louis, MO) for 2 h and exposed to 10 ng/ml of DNP-labeled human serum albumin (Sigma) at 37°C for 30 min. The supernatants of the culture (50 µl) was transferred into a 96-well microtitre plate (Becton Dickinson) and mixed with 50 µl of 1 mM *p*-nitrophenyl-*N*-acetyl- β -D-glucosaminide (Sigma) in a 0.1 M citrate buffer (pH 4.5) at 37°C for 1 h. After the incubation, the reaction was terminated by adding 200 µl of a 0.1 M sodium carbonate buffer (pH 10.0). The absorbance of the mixture at 405 nm was measured with a microplate reader (model 550; Bio-Rad, Hercules, CA). The relative value of the β -hexosaminidase activity was calculated by the following formula: inhibition (%) = $\{1 - (T - N)/(P - N)\} \times 100$, where T, P, and N respectively represent the absorbance of equal portions of the supernatant from quince HW-treated cells upon degranulation, control (untreated) cells upon degranulation, and control cells without the degranulation treatment with antigen alone. In parallel with this assay, the growth and viability of the cells in the presence or absence of quince HW for 120 h was evaluated by counting the cells with a hemacytometer after staining with trypan blue.

Reverse transcription polymerase chain reaction (RT-PCR). RBL-2H3 cells were cultured at 1×10^6 cells/ml in an RPMI 1640 medium containing 10% FBS with or without 50, 100, or 200 µg/ml of quince HW for 24 h. After this cultivation, total RNA was extracted from the cells by using the TRIzol reagent (Invitrogen) according to the manufacturer's instructions. The RNA (1 µg) was reverse-transcribed into cDNA with 1 mM each of dNTP, 10 pmol/µl of oligo(dT)₁₈ primers, and 25 units/µl of M-MLV reverse transcriptase (Invitrogen) at 42°C for 50 min, using a PTC-200 DNA Engine (MJ Research, Waltham, MA). All PCRs were performed with a Taq PCR Core kit (Qiagen, Chatsworth, CA) and 10 pmol/ml of the primers by using PTC-200. The primer sequences used in this study were as follows: FcεRI α (sense), 5'-GGAACAATTCCTCTCAAATG-3', and FcεRI α (antisense), 5'-GGGTACAGACATTTCTATGG-3' (product size 729, accession number M17153); FcεRI β (sense), 5'-GGCAACATTTCTGTTTCCTC-3', and FcεRI β (antisense), 5'-GCATCTCACTACACTGAATG-3' (product size 564, accession number M22923); FcεRI γ (sense), 5'-GGAGAAGAGAAATGTCAGTG-3', and FcεRI γ (antisense), 5'-GCTCTGCTATATCCTGGATG-3' (product size 381, accession number BC160864); and β -actin (sense), 5'-TGTATG-CCTCTGGTCCGTACCAC-3', and β -actin (antisense), 5'-ACAGAG-TACTTGCGCTCAGGAG-3' (product size 592, accession number BC063166).¹⁶ The following program was used: 94°C for 1 min, 62°C for 1 min, and 72°C for 2 min, 30 cycles for FcεRI α ; 94°C for 1 min, 60°C for 1 min, and 72°C for 2 min, 28 cycles for FcεRI β ; 94°C for 1 min, 60°C for 1 min, and 72°C for 2 min, 30 cycles for FcεRI γ ; 94°C for 30 s, 64°C for 1 min, and 72°C for 1 min, 18 cycles for β -actin. After the amplification, 10 µl of the reaction mixture was electrophoresed on 2.0% agarose gel and the PCR products were visualized as bands by ethidium bromide staining for 20 min. Gel images were captured with a Printgraph AE-6911FX device (Atto, Tokyo, Japan).

Statistical analysis. Statistical analysis of the data employed a one-tailed and two-tailed Student's *t*-test for *in vivo* and *in vitro* studies, respectively. A *p*-value of less than 0.05 was accepted as being statistically significant.

Results

Skin severity

Hot-water extraction yielded 173 g of quince HW from approximately 3,200 g of quince fruits. The effect of the oral administration of the quince HW-added diet

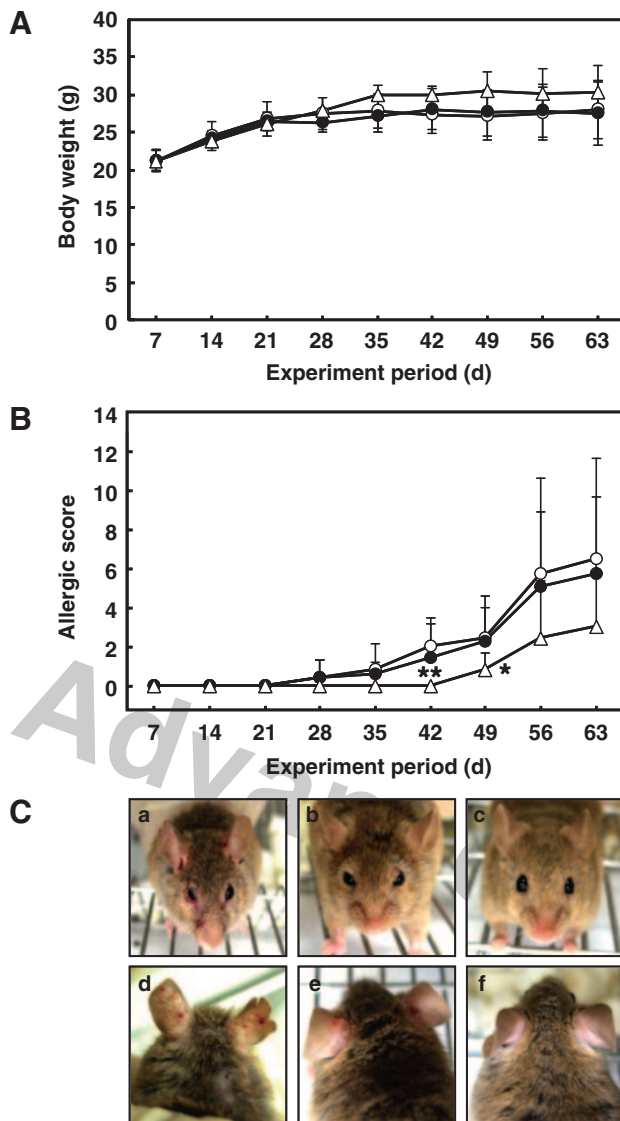


Fig. 1. Effect of the Oral Administration of Quince HW-Supplemented Diet on NC/Nga Mice.

A, Effect of quince HW on body weight. Data are represented as the mean value $\pm$ standard deviation (SD) of body weights of the control-diet group ($\text{---}\bigcirc\text{---}$), 2.5% quince HW-diet group ($\text{---}\bullet\text{---}$), and 5.0% quince HW-diet group ($\text{---}\triangle\text{---}$) ($n = 5$). B, Effect of quince HW on skin severity. Data are represented as the mean value $\pm$ SD of the allergic scores of the control-diet group ($\text{---}\bigcirc\text{---}$), 2.5% quince HW-diet group ($\text{---}\bullet\text{---}$), and 5.0% quince HW-diet group ($\text{---}\triangle\text{---}$) ($n = 5$). * $p < 0.05$ and ** $p < 0.01$ vs. the control group. C, Clinical features of AD-like skin lesions on the face and ear auricle on d 53; a and d, control-diet group; b and e, 2.5% quince HW-diet group; and c and f, 5.0% quince HW-diet group.

on NC/Nga mice is shown in Fig. 1. Throughout the experimental period, no significant difference in the change of average body weight was apparent among the groups (Fig. 1A). Five AD-like signs and symptoms appeared clinically on the face, ear, nose, neck, and dorsal skin of mice fed with the control diet at around d 21 or later of the experiment and developed gradually with aging. The skin severity scores of the 2.5% and 5.0% quince HW-fed group tended to be lower than the control group. The 5.0% quince HW-fed group showed no apparent skin lesions until d 42 and significantly ($p < 0.01$) alleviated grades of dermatitis on d 42 and d 49 compared to the control diet group (Fig. 1B). The

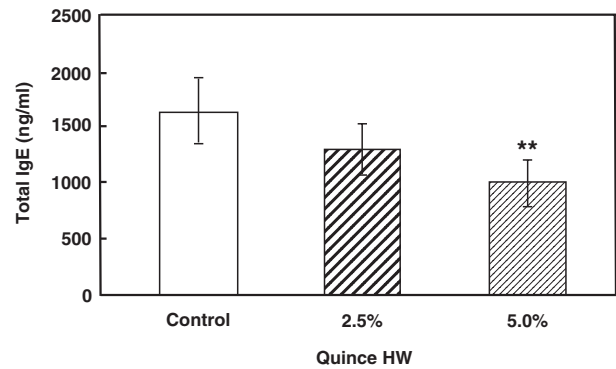


Fig. 2. Serum IgE Concentration in the NC/Nga Mice.

The IgE concentrations of the serum obtained from the control-diet group, 2.5% quince HW-diet group, and 5.0% quince HW-diet group are shown. Data are represented as the mean value $\pm$ SD ($n = 5$). ** $p < 0.01$ vs. the control-diet group.

alleviated clinical features of the symptoms were observed, particularly on the face and ears of the mice in both quince HW-added diet groups compared with the control group (Fig. 1C). The scratching behavior of the 5.0% quince HW diet group tended to be less than that of the control group (data not shown).

Serum IgE level

Serum was separately collected from control diet group, 2.5% quince HW-added diet group, and 5.0% quince HW-added diet group on d 63. The levels of IgE in the serum were as follows: control group, 1635 ± 289 ; 2.5% quince group, 1300 ± 226 ; and 5.0% quince group, 994 ± 205 ng/ml (Fig. 2). Quince HW reduced the IgE level in a dose-dependent manner. In particular, the 5.0% quince HW-added diet group showed a highly significant value ($p < 0.01$).

Beta-hexosaminidase assay

The effect of quince HW on the β -hexosaminidase release from RBL-2H3 cells is shown in Fig. 3. After 24 h, quince HW at a concentration of 50, 100, and 200 μ g/ml inhibited 23.0%, 27.4%, and 12.8% of the β -hexosaminidase release, respectively. The proliferation and viability of RBL-2H3 treated by quince HW was no different from those under the quince HW-free condition (Fig. 3B and C).

Expression of Fc ϵ RI subunits

The expression of Fc ϵ RI subunits in RBL-2H3 is shown in Fig. 4. Treatment with quince HW showed no effects on the expression of the Fc ϵ RI α and β subunits. In contrast, the expression of the γ subunit was notably reduced (Fig. 4A). The fraction of quince HW weighing less than 3 kDa showed reduced expression of the Fc ϵ RI γ subunit, while that of more than 3 kDa had a negligible effect (Fig. 4B).

Discussion

The anti-allergic effect of quince fruit has not been reported despite its useful application against sore throats and coughs. In this study, we revealed the novel inhibitory effects of quince HW on AD-like symptoms in NC/Nga mice. As shown in Fig. 1, the development

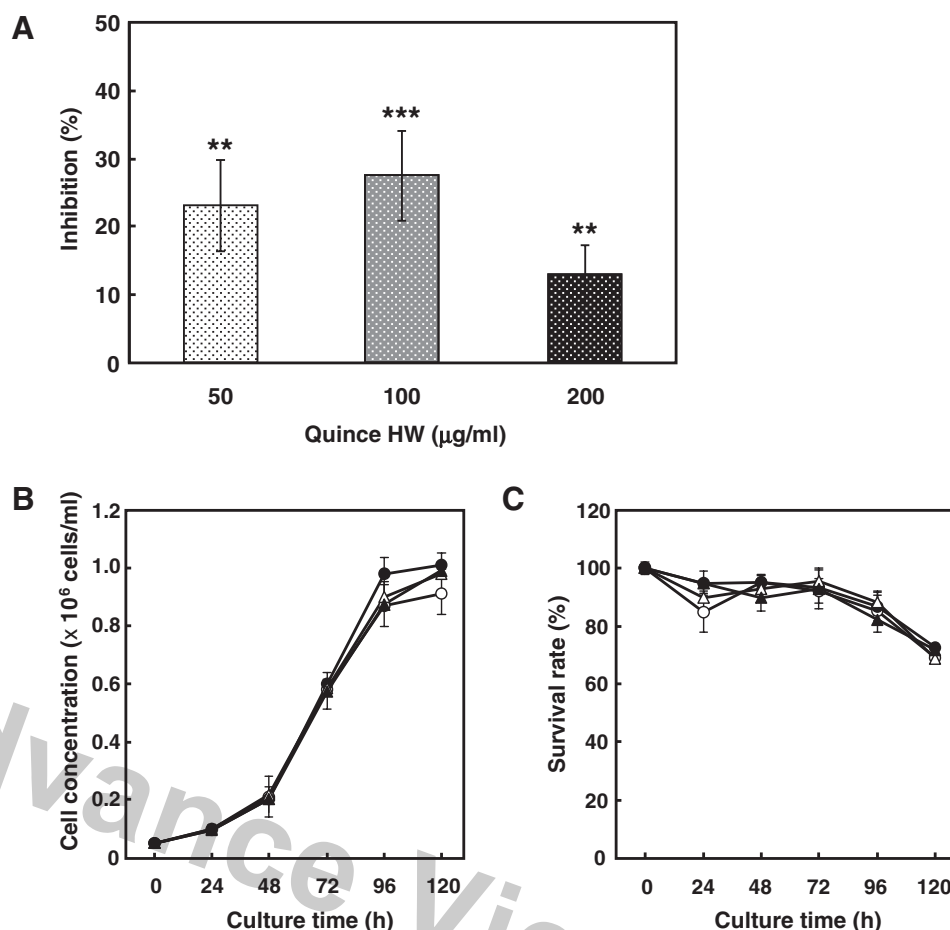


Fig. 3. Effect of Quince HW on the β -Hexosaminidase Release from RBL-2H3.

A, Effect of quince HW on β -hexosaminidase release from RBL-2H3. Data are represented as the mean value $\pm$ SD ($n = 3$). ** $p < 0.01$ and *** $p < 0.001$ vs. cell cultures without a sample. B, Effect of quince HW (0, 50, 100, and 200 $\mu\text{g/ml}$) on cell growth of RBL-2H3. Data are represented as the mean value $\pm$ SD of the cell concentration in the presence of none ($\text{---}\bigcirc\text{---}$), 50 $\mu\text{g/ml}$ ($\text{---}\bullet\text{---}$), 100 $\mu\text{g/ml}$ ($\text{---}\triangle\text{---}$), and 200 $\mu\text{g/ml}$ ($\text{---}\blacktriangle\text{---}$) of quince HW ($n = 3$). C, Effect of quince HW (0, 50, 100, and 200 $\mu\text{g/ml}$) on the cell viability of RBL-2H3. Data are represented as the mean value $\pm$ SD of the survival rate in the presence of none ($\text{---}\bigcirc\text{---}$), 50 $\mu\text{g/ml}$ ($\text{---}\bullet\text{---}$), 100 $\mu\text{g/ml}$ ($\text{---}\triangle\text{---}$), and 200 $\mu\text{g/ml}$ ($\text{---}\blacktriangle\text{---}$) of quince HW ($n = 3$).

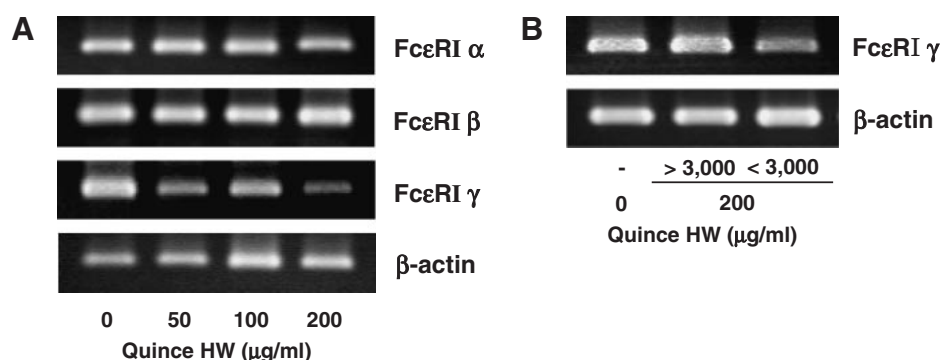


Fig. 4. Effect of Quince HW on mRNA Expression of Fc ϵ RI Subunits in RBL-2H3.

A, Intracellular mRNA expression of Fc ϵ RI subunits and β -actin after treating or not with quince HW. B, Intracellular mRNA expression of Fc ϵ RI γ subunit β -actin after treating or not with fractionated quince HW.

of skin severity was significantly reduced in the mice fed with the 5.0% quince HW-added diet. The serum IgE level of the quince HW-added diet groups was lowered in a dose-dependent manner (Fig. 2). NC/Nga mice are known to exhibit spontaneous development of dermatitis accompanied by IgE elevation with age under conventional circumstances.^{17,18} Many reports have demonstrated that an elevated level of IgE antibodies played an

important role in the development of AD-like skin lesions in NC/Nga mice.^{17,18} Furthermore, elevation of the serum IgE level has been reported to be correlated with the degranulation of mast cells and eosinophils.¹⁷ Our results suggest that the oral administration of quince HW inhibited AD-like skin lesions by an unreported IgE-suppressing mechanism. However, it has also been reported that the development of some chronic AD-like

skin lesions in NC/Nga mice was independent of the plasma IgE level.^{19–21} These facts led us to further investigate the distinct inhibitory effect of quince HW on the development of allergic symptoms.

Figure 3 shows that the β -hexosaminidase release from RBL-2H3 cells was significantly reduced by treating with quince HW without affecting the proliferation and viability of the cells. The value for released β -hexosaminidase has frequently been used as an indicator for evaluating the extent of degranulation by mast cells or basophils, because this exoglycosidase is stored in the secretory granules of these cells and released in parallel with chemical mediators by Fc ϵ RI-mediated activation.^{22,23} The decreased β -hexosaminidase activity suggests that quince HW inhibited the degranulation process itself.

We further found that a 24-h treatment with quince HW reduced the expression of the Fc ϵ RI γ subunit (Fig. 4A). Fc ϵ RI is expressed exclusively on mast cells and basophils for triggering the degranulation. Fc ϵ RI is expressed in mice as a tetrameric molecule consisting of one α chain, one β chain, and two disulfide-linked γ chains.^{24,25} IgE-binding and signal-transduction of the Fc ϵ RI molecule are performed by distinct subunits.^{24,26} The Fc ϵ RI α subunit mostly extends out to the extracellular region, binding to the constant region of IgE with high affinity to begin a cascade of cellular events.²⁷ The Fc ϵ RI β subunit amplifies the signal strength.²⁸ The Fc ϵ RI γ subunit enables the stable expression of the Fc ϵ RI α subunit on the cell surface and transfers the signal to induce degranulation via immunoreceptor tyrosine activation motifs (ITAM).^{25,29} Therefore, it is possible that reduced expression of the Fc ϵ RI γ subunit was a factor in the inhibition of degranulation by quince HW.

We characterized the active substance in quince HW for suppressing the Fc ϵ RI γ subunit. Fc ϵ RI γ subunit expression was suppressed by the filtered (<3 kDa) fraction (Fig. 4B), suggesting that a low-molecular-weight substance was involved in down-regulation of the Fc ϵ RI γ subunit. To date, many low-molecular-weight polyphenols have been reported as anti-allergic substances that inhibited the degranulation process.^{30–35} Some of these polyphenols are known to suppress class-switching from IgM to IgE in B cells or the expression of Fc ϵ RI α and γ subunits in mast cells.^{36–38} Quince fruit has been reported to contain low-molecular-weight polyphenols such as caffeoylquinic acids (3-, 4-, and 5-caffeoylquinic acids and 3,5-dicaffeoylquinic acid), quercetin glycosides (quercetin-3-galactoside and quercetin-3-rutinoside), and kaempferol glycosides (kaempferol-3-glucoside and kaempferol-3-rutinoside) in the pulp, peel, and seeds.^{1,39,40} We have also identified 3-, 4-, and 5-caffeoylquinic acid as the major monomeric polyphenols in quince HW by a reverse-phase high-performance liquid chromatographic analysis, as reported for the methanol or aqueous acetone extract of quince fruit (data not shown).² Some of the polyphenols found in quince fruit have been reported to have anti-inflammatory effect, including the inhibition of histamine release from mast cells.^{41–45} These low-molecular-weight polyphenols are therefore suggested to exhibit have had the anti-allergic effects observed in this study. However, it should be noted that anti-inflamma-

tory polyphenols which inhibit Fc ϵ RI γ chain expression and IgE production have been found in limited sources other than quince fruits and have a specific structure distinct from the above-mentioned quince fruit-derived polyphenols. Other unknown polyphenolic or other substances may be involved in the suppressive effect of quince HW on type I allergic symptoms.

In conclusion, we found two anti-allergic effects of quince HW *in vivo* and *in vitro*. The inhibitory effects of quince fruit on both the IgE elevation in allergic mice and Fc ϵ RI γ chain expression in mast cells and basophils have not been reported in previous studies. Although further detailed studies are necessary to understand the anti-allergic mechanism of quince HW, our results suggest that quince HW would be applicable as a functional food for alleviating type I allergic symptoms.

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